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GENETIC CHARACTERISTICS OF HEALTHY AND DECLINING SUGAR MAPLE (ACER SACCHARUM MARSH) IN ONTARIO

R. A. C. PROJECT NO. 435G
FINAL REPORT

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FINAL REPORT

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ABSTRACT

Potential genetic differences between healthy and declining sugar maple (*Acer saccharum* Marsh.) were investigated at four sites in Ontario. Eleven polymorphic enzyme loci were assayed using starch-gel electrophoresis and the genetic data was analyzed statistically in conjunction with decline index (DI) ratings of individual trees. Significant allelic heterogeneity between healthy and declining subsamples was observed at one locus (*Mdh-3*) at one site (Davies), but there was no evidence of direct involvement of *Mdh-3* in resistance or susceptibility to decline. Levels of expected heterozygosity of healthy and declining subsamples were also significantly different at the Davies site with the declining subsample demonstrating greater heterozygosity. Results from the other sites were contradictory but not statistically significant. No trend was apparent concerning observed heterozygosity. Comparisons of allele frequencies and genetic diversities among healthy and declining subsamples of an individual site may be confounded by cohort associations or family structure. Therefore, it is desirable to limit such structure when sampling, but this may be difficult in natural populations where parentage is unknown. Fixation indices (F_{IS}) were consistently greater in declining subsamples than in the healthy subsample of the same site suggesting that the proportion of heterozygous loci per individual relative to Hardy-Weinberg expectations tends to be greater in the healthy individuals. Such a relationship could occur if inbreeding leads to a predisposition to decline and/or if individual heterozygosity contributes to resistance to decline. The relative contribution of either of these possible conditions to the observed trend in F_{IS} is not easily discerned.

INTRODUCTION

Over the past few years forest decline has attracted global concern. Largely enigmatic in origin, it has been described throughout much of North America and Europe (Hornbeck and Smith, 1985; Knight, 1987; Linzon, 1985; Schütt and Cowling, 1985). The changing chemical climate has received considerable attention as a probable contributing factor. It is generally agreed that air pollutants such as ozone and oxides of nitrogen and sulphur accompanied by an increase in acidity of precipitation are detrimental to the world's forests (Anderson, 1989; Linzon, 1985; Morrison, 1984; Schulze, 1989). Although acidic deposition has often been singled out as a primary malefactor, it is unlikely that it alone is the major cause of forest decline (Pitelka and Raynal, 1989; Roberts *et al.*, 1989; Skelly, 1989). Decline may be the result of the interactions of a number of stresses of which pollution may be one part (Bardy, 1989; Linzon, 1986; Mueller-Dombois, 1988; Pierre and Queiroz, 1988). In some species, decline may be a stage in a normal process initiated by periodic natural disturbances such as severe drought (Hosking and Hutcheson, 1988) or may be a response to long term climatic shifts (Hamburg and Cogbill, 1988). Whatever its cause, the problem of forest decline is real and widespread.

Among the Canadian species evoking the greatest concern is sugar maple (*Acer saccharum* Marsh.). Reports of maple decline in Ontario began as early as 1947 and many of those which have followed have listed its cause as unknown (McIlveen *et al.*, 1986). It is not surprising that maple syrup producers have blamed acid rain for the decline of their sugar bushes since acidic deposition in southern Ontario is among the greatest in North America (Wisniewski and Kinsman, 1988), but indications are that acidic precipitation is but one more stress in addition to those of insect outbreaks and drought (Hendershot and Jones, 1989; Linzon, 1986; McLaughlin *et al.*, 1985). According to Hendershot and Jones (1989) areas with poor soils and marginal climate are predisposed to decline.

Previous research in forest trees, which has primarily considered air pollution to be the causal agent, has provided reason to believe that genetic differences may exist between healthy and deteriorating individuals. Variation in tolerance levels to pollutants has been recorded among provenances in *Pinus strobus*, (Kriebel and Leben, 1981), *P. sylvestris* (Huttunen, 1978), and *Picea abies* (Huttunen, 1978; Scholz and Venne, 1989), and among clones in *Picea abies* (Scholz and Venne, 1989), *Pinus strobus* (Houston and Stairs, 1973), and *Populus tremuloides* (Wang *et al.*, 1986). Concern about the potential erosion of genetic resources through the elimination of sensitive individuals has been expressed (Karnosky *et al.*, 1989; Kreibel and Leben, 1981; Scholz, 1981). The purpose of the present study was to determine whether possible genetic differences between healthy and declining sugar maple within the same sites are discernable at the allozyme level. Regardless of the specific causal agents, the determination of any link between genetic markers and visible injury could provide a foundation for selection and management decisions.

MATERIALS AND METHODS

Data Collection

The sugar maple examined in this study were from four sites (Figure 1) that had been considered in an earlier study (Beak Consultants Ltd., 1989). At each site, bud tissue was collected from 24 sugar maple trees previously classed as healthy and from 24 trees (23 at Boothby) which had been classed as declining, giving a total sample size of 47 to 48 trees per site. Bud tissue was prepared, and starch-gel electrophoresis was carried out as previously described (Perry and Knowles, 1989). A total of nine enzyme systems were assayed (Table 1). Only variable loci (eleven in total) were considered and although *Sod-2* was found to have low level variability in earlier studies (Lelliot, 1987; Perry and Knowles, 1989), it was invariant in the presently sampled trees. Therefore it was excluded from further analysis.

FIGURE 1. LOCATIONS OF FOUR SUGAR MAPLE SITES SAMPLED IN ONTARIO.

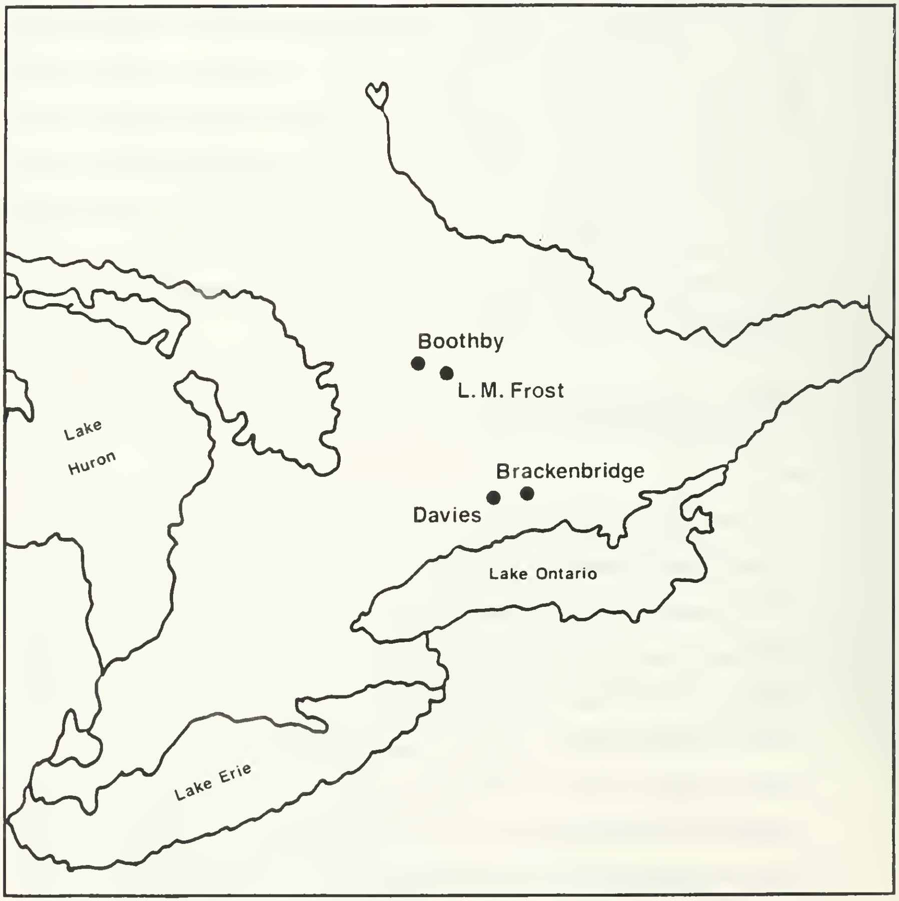

TABLE 1. ENZYME SYSTEMS ASSAYED IN BUD TISSUE OF SUGAR MAPLE.

ENZYME SYSTEM	E.C. DESIGNATION	BUFFER SYSTEM[a]
Aspartate aminotransferase (AAT)	2.6.1.1	B
Aldolase (ALD)	4.1.2.13	H
Diaphorase (DIA)	1.6.4.3	B
Leucine-amino peptidase (LAP)	3.4.11.1	B
Malate dehydrogenase (MDH)	1.1.1.37	H
6-Phosphogluconic dehydrogenase (6PG)	1.1.1.44	H
Phosphoglucose isomerase (PGI)	5.3.1.9	B
Phosphoglucomutase (PGM)	2.7.5.1	H
Superoxide dismutase (SOD)	1.15.1.1	B

[a] Buffer systems as described by Cheliak and Pitel (1984).

A decline index (DI) was available for each sampled tree. This index was based on the percentage of dead branches in the tree crown, the percentage of slight and/or strong chlorosis, and the percentage of undersized leaves (McIlveen and McLaughlin, 1988). The DI values adopted were the averages of estimates made by two observers.

Statistical Analysis

Allele frequencies were estimated for both subsamples of each population based on the observed genotypic arrays. In many cases, small expected numbers (<5) precluded valid testing of allelic heterogeneity using standard chi-square or *G*-tests. Therefore, heterogeneity among allele frequency estimates within sites was evaluated via Monte Carlo methods as follows. Using a computer program, for each site and each locus, a genotypic distribution equivalent to the pooled observed healthy and declining samples was randomly divided into two synthetic classes. The proportion of 10,000 such synthetic divisions having allele frequencies equally or more unevenly distributed than those observed was considered as the probability of the observed distribution.

Expected heterozygosities (H_e) were estimated for each locus within each subsample as:

$$H_e = 1 - \Sigma p_i^2,$$

where p_i was the estimated frequency of the ith allele at the locus. A measure of the deviation of observed heterozygote frequency from that expected under Hardy-Weinberg equilibrium, F_{IS} was calculated for each subsample as:

$$F_{IS} = 1 - H_o/H_e,$$

where H_o was the observed proportion of heterozygotes. The contribution of each locus to a subsample's $\bar{F}_{IS}$ was weighted by its expected heterozygosity in that subsample. Values of F_{IS} greater than 0 indicate an excess of homozygous genotypes relative to Hardy-Weinberg expectations while negative values are indicative of an excess of heterozygotes. For each site, subsample differences in $\bar{H}_e$, $\bar{H}_o$, and $\bar{F}_{IS}$ were evaluated using paired t-tests.

Some overlap of DI between subsamples within three of the four sites was noted. For example, the healthy subsample at the Boothby site had DI values ranging from 1.87 to 17.39 while the declining subsample had trees with DI estimates ranging from 12.14 to 44.84. To remove possible effects of this overlap, all trees within a site were ordered according to their DI and the twenty trees with the highest DI within a site were considered as a new declining subsample and the twenty trees with the lowest DI within that site were reclassified as a new healthy subsample. Herein these new subsamples are referred to as reduced data. The statistical analyses described above were performed both on these reduced data and on the data of subsamples as originally designated (original data).

The possibility of a relationship between heterozygosity and vigour was explored through a regression analysis of individual tree data. The independent variable was the proportion of loci heterozygous in a tree and the dependent variable was the tree's DI. This analysis was performed for each site using the computer program MCREG of BIOΣTAT I (Pimentel and Smith, 1985).

RESULTS

Observed allele frequencies of the healthy and declining subsamples (original data) within the four sites are presented in Table 2. Monte Carlo methods

TABLE 2. ALLELE FREQUENCIES OF HEALTHY (H) AND DECLINING (D) SUGAR MAPLE AT FOUR SITES (ORIGINAL DATA).

		SITE[1]							
		BOTH		BRAK		DAV		LMF	
LOCUS	ALLELE	H	D	H	D	H	D	H	D
Aat-1	1	0.98	0.94	0.96	0.96	1.00	0.92	1.00	0.96
	2	0.02	0.02	0.04	0.04	0.00	0.06	0.00	0.02
	3	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.02
	4	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Aat-2	1	0.69	0.85	0.63	0.73	0.90	0.79	0.79	0.83
	2	0.31	0.15	0.31	0.25	0.08	0.21	0.21	0.17
	3	0.00	0.00	0.06	0.02	0.02	0.00	0.00	0.00
Ald-3	1	0.90	0.89	0.98	0.90	1.00	0.92	0.90	0.94
	2	0.10	0.09	0.02	0.10	0.00	0.08	0.08	0.06
	3	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Dia-3	1	0.88	0.96	0.96	0.92	0.96	0.92	0.85	0.92
	2	0.08	0.04	0.04	0.08	0.04	0.08	0.13	0.06
	3	0.04	0.00	0.00	0.00	0.00	0.00	0.02	0.02
Lap-1	1	0.98	0.98	1.00	0.94	1.00	1.00	1.00	1.00
	2	0.02	0.02	0.00	0.06	0.00	0.00	0.00	0.00
Lap-2	1	0.96	1.00	0.98	0.98	1.00	1.00	1.00	0.98
	2	0.04	0.00	0.02	0.02	0.00	0.00	0.00	0.02
Mdh-3	1	0.92	1.00	0.94	0.96	0.98	0.85*	0.96	0.96
	2	0.08	0.00	0.06	0.04	0.02	0.15	0.04	0.02
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
6pg-1	1	1.00	1.00	0.98	1.00	1.00	0.98	0.96	1.00
	3	0.00	0.00	0.02	0.00	0.00	0.02	0.04	0.00
Pgi-2	1	0.88	0.76	0.73	0.79	0.73	0.67	0.85	0.79
	2	0.06	0.17	0.21	0.19	0.08	0.15	0.13	0.10
	3	0.04	0.04	0.02	0.00	0.15	0.06	0.02	0.04
	4	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
	5	0.02	0.02	0.02	0.02	0.04	0.12	0.00	0.06
Pgm-1	1	0.90	0.91	0.96	0.90	0.81	0.88	0.90	0.88
	2	0.00	0.02	0.02	0.02	0.02	0.04	0.04	0.00
	3	0.10	0.07	0.02	0.08	0.17	0.08	0.06	0.12
Pgm-2	1	0.98	1.00	1.00	0.98	1.00	0.98	1.00	0.96
	2	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02
	3	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.02

[1] Site names: BOTH=Boothby; BRAK=Brackenbridge; DAV=Davies; LMF=L.M. Frost.

*Significant ($P<0.05$) difference between subsample allele frequencies within site.

demonstrated that heterogeneity was significant (P=0.04) at *Mdh-3* in the Davies site. Allele frequencies in the reduced data (not presented) varied little from those of the original data and conclusions regarding heterogeneity were unaltered.

Mean DI of subsamples within sites ranged from 5.72 to 30.98 in the original data. When the reduced data were viewed, this range increased from 4.74 to 33.55. In general, declining subsamples had mean DI about 4 times greater than the corresponding healthy subsamples using original data (Table 3). When reduced data were considered, the DI of declining subsamples were just over 5 times greater on average.

TABLE 3. MEAN DAMAGE INDEX OF HEALTHY AND DECLINING SUGAR MAPLE AT FOUR SITES (ORIGINAL DATA).

SITE	HEALTHY	DECLINING
BOOTHBY	7.40	25.54
BRACKENBRIDGE	5.72	27.45
DAVIES	8.55	30.98
L.M. FROST	6.72	29.86
POOLED SITES	7.10	28.49

Paired t-tests indicated a significant difference in the levels of expected heterozygosity of the healthy and declining subsamples within the Davies site. The declining subsample had a greater $\bar{H}_e$ in both the original and reduced data (Table 4). However, in the remaining sites $\bar{H}_e$ was usually larger, but not significantly so, in the healthy subsamples. No consistent trend was noted concerning a possible relationship between DI and observed heterozygosity and none of the sites demonstrated significant heterogeneity of $\bar{H}_o$ among subsamples (Table 5).

TABLE 4. EXPECTED HETEROZYGOSITIES PER LOCUS ($\bar{H}_e$) OF HEALTHY AND DECLINING SUGAR MAPLE AT FOUR SITES.

SITE	ORIGINAL DATA HEALTHY	ORIGINAL DATA DECLINING	REDUCED DATA HEALTHY	REDUCED DATA DECLINING
BOOTHBY	0.17	0.13	0.18	0.14
BRACKENBRIDGE	0.16	0.17	0.17	0.16
DAVIES	0.12	0.19*	0.12	0.19*
L.M. FROST	0.16	0.15	0.15	0.15
POOLED DATA	0.16	0.16	0.16	0.16

*Estimates differ significantly ($P<0.05$) between subsamples within site.

TABLE 5. OBSERVED HETEROZYGOSITIES PER LOCUS ($\bar{H}_o$) OF HEALTHY AND DECLINING SUGAR MAPLE AT FOUR SITES.

SITE	ORIGINAL DATA HEALTHY	ORIGINAL DATA DECLINING	REDUCED DATA HEALTHY	REDUCED DATA DECLINING
BOOTHBY	0.18	0.14	0.19	0.14
BRACKENBRIDGE	0.18	0.16	0.19	0.15
DAVIES	0.11	0.17	0.12	0.17
L.M. FROST	0.17	0.16	0.15	0.15
POOLED DATA	0.16	0.16	0.17	0.15

Regardless of the direction of the shift in $\bar{H}_e$ or $\bar{H}_o$ among subsamples within a site, $\bar{F}_{IS}$ was consistently greater in the declining subsamples than in the corresponding healthy subsamples (Table 6). In addition, when compared to estimates obtained using original data, in three of the four sites $\bar{F}_{IS}$ of healthy subsamples was smaller (more negative) and that of declining subsamples was larger when made with reduced data, indicating a possible strengthening of the trend when the overlap within the original data was removed. Although paired *t*-tests did not find any of the observed differences in $\bar{F}_{IS}$ within sites to be statistically significant, the apparent trend was compelling.

TABLE 6. FIXATION INDICES ($\bar{F}_{IS}$) OF HEALTHY AND DECLINING SUGAR MAPLE AT FOUR SITES.

	ORIGINAL DATA		REDUCED DATA	
SITE	HEALTHY	DECLINING	HEALTHY	DECLINING
BOOTHBY	-0.054	-0.036	-0.083	-0.020
BRACKENBRIDGE	-0.130	0.075	-0.169	0.098
DAVIES	0.041	0.095	-0.008	0.101
L.M. FROST	-0.066	-0.012	-0.028	-0.020
POOLED DATA	-0.022	0.050	-0.044	0.063

Not unexpectedly, the results of the regression analysis reflected the observations made of $\bar{H}_o$ above. The possibility of a relationship between heterozygosity and state of decline was strongest in the Davies site with a correlation (r) between individual heterozygosity and DI of 0.27 (P=0.06). The Boothby, Brackenbridge and L.M. Frost sites had r values of -0.22 (P=0.13), -0.21 (P=0.16), and 0.05 (P=0.74) respectively. Clearly there was no consistent trend.

DISCUSSION

Several observations made in the course of this study are of interest. First, where others report increased heterozygosity in forest trees tolerant of environmental stress (Geburek *et al.* 1987; Müller-Starck, 1985, 1989) we found no evidence of such a trend either in comparison of mean heterozygosity measures of subsamples or in the regression analyses. In contrast, the only statistically significant relationship observed was contradictory with a greater $\bar{H}_e$ found in a the declining subsample of the Davies site. But this trend was not observed in data of the other sites. It should be noted that not all researchers have found a clear relationship between heterozygosity and apparent tolerance to environmental stress in forest trees (Bergmann and Scholz, 1989).

The Davies site was also unique in that significant allelic heterogeneity was observed among its subsamples while no such heterogeneity was detected for the other sites. In other forest trees, heterogeneity of allele frequencies has been observed among groups sensitive or tolerant to environmental stress (Bergmann and Scholz, 1985, 1989; Geburek *et al.*, 1987; Müller-Starck, 1985, 1989; Scholz and Bergmann, 1984) and the researchers involved have often suggested that the specific enzymes may be involved in resistance mechanisms or are acted upon through genic selection. We have no evidence that the *Mdh-3* locus is directly involved in resistance or susceptibility to sugar maple decline. Note that the distribution of allele *2* at *Mdh-3* in the Boothby site is opposite to that observed at the Davies site. Therefore, if the decline occurring at both sites is attributable to the same stress factors, *Mdh-3* cannot be directly involved with a resistance mechanism or in some other manner be a specific target of selection.

Differences in allele frequencies between tolerant and sensitive phenotypes in this and similar allozyme investigations could be accounted for by influences other than selection imposed by environmental stress. We suspect that the allelic heterogeneity observed between healthy and declining subsamples at the Davies site could be a reflection of the population's genetic architecture. Sugar maple stands may be composed of a number of cohorts (progeny produced during the same year) which may be genetically differentiated (Mulcahy, 1975). Willis and Coffman (1975) suggest that stands may be spatially contagious with respect to age. In addition, through allozyme analysis of three northern sugar maple stands, we have found evidence of spatial genetic structure that we interpret to be a reflection of cohorts or family groups (manuscript in preparation). Therefore, if cohorts or family groups that differ in their allozyme characteristics also differ in their resistance to pollution or other stress factors, significant differences in allozyme frequencies or diversities between tolerant and sensitive groups may result. These allozyme differences would likely not be the result of direct action of environmental stress, but would be artifacts of cohort association.

Allelic heterogeneity observed among tolerant and sensitive subsets in some earlier studies might be explained in a similar manner. For example, where seed is collected from only a small number of trees and the resulting progeny are tested for resistance to a particular agent (eg., SO_2), it would not be unexpected that the tolerant subset would be dominated by members of a few specific families and the sensitive subset by members of a few others, if resistance is a heritable trait. At the same time, allozyme differences are expected among families although these differences need not be associated with resistance. Clearly it is important to limit family structure within test populations in order to avoid possible confounding effects. This is difficult in natural populations where parentage is unknown.

Some researchers have expressed concern that some alleles are only represented in the sensitive subpopulations that they have studied and thus are considered endangered (Bergmann and Scholz, 1985, 1987, 1989; Geburek *et al.*, 1987). Considering the sample sizes, the present study does not provide evidence that any of the observed alleles are at risk. While several alleles were found exclusively in the declining subsamples, their frequencies were low. Actual overall occurrence of the most frequent of such subsample specific alleles (allele *3* at *Aat-1*) was in three heterozygotes out of the total sample of 191. This is not convincing evidence of an allelic association with sensitivity and imminent loss of an allele since the probability that all three of the observed heterozygotes would be grouped together in the same decline index class by chance alone is approximately 0.12.

What inferences can be drawn from our findings? Perhaps the most notable trend observed was that of F_{IS}. That all four populations displayed greater F_{IS} in declining subsamples (Table 6) may not be convincing on its own. However, when subsample composition was redefined in accordance with DI (reduced data) there was a concurrent decrease of F_{IS} in healthy subsamples and increase of F_{IS} in declining subsamples for three of the four populations. We regard this as evidence that the perceived trend is something more than an ephemeral aberration.

This trend in F_{IS} indicates that the proportion of heterozygous loci per individual relative to Hardy-Weinberg expectations tends to be greater in the healthy individuals. Such a condition could occur if the more inbred individuals are predisposed to decline, or if heterozygosity in general confers resistance to decline, or both. It is difficult to distinguish between these possibilities (Mitton and Jeffers, 1989). That the significant difference in heterozygosities observed among subsamples at the Davies site had the declining subsample with the highest heterozygosity would suggest that heterozygosity *per se* does not necessarily impart resistance. However, if all inbred individuals were eliminated, the lowest F_{IS} value expected would be 0 unless other factors were involved. Lower F_{IS} values in healthy or tolerant subsamples might tend to lead to higher heterozygosities in those subsamples than in declining or sensitive subsamples, but not always as evidenced by the Davies site. If one were to interpolate these observations concerning F_{IS} to a breeding program with a goal of reducing susceptibility to decline, one might select superior parents based on field observations, and then make selections within families according to allozyme heterozygosity. However, it should be noted that further study is required to confirm the proposed trend.

A question that remained following an earlier study (Perry and Knowles, 1989) was whether the levels of variation observed in five sugar maple populations at the northern range limit in Ontario was representative of the species. Although the data of the present study were not collected specifically to address this question and the sampling methods used could introduce some bias, we can make some tentative observations. In the northern populations previously studied, $\bar{H}_e$ ranged from 0.098 to 0.132 per population with an overall mean of 0.110. In the present study, if the same loci which include five monomorphic loci (*Mdh-1* and *2*, *Sod-1*, *2*, and *3*) are considered, $\bar{H}_e$ ranges from 0.103 to 0.128 with an overall mean of 0.115 (data not presented). Therefore, there is no evidence that the marginal populations are less variable than central ones as has been suggested by studies in other forest tree species (Yeh and Layton, 1979; Guries and Ledig, 1982).

This investigation raises many new questions. Is there variation in the level of decline among cohorts within sites? Obviously tree ages would have to be considered to address this question. Are declining and healthy individuals distributed randomly within sites? This would require that tree positions be mapped so that statistical procedures such as spatial autocorrelation analysis could be applied. If the decline has a spatial element, is it related to genetic or environmental factors? How might artifacts of family structure or cohort association be minimized when comparing declining and healthy subsamples from natural stands? As is the case with the study of interactions of environmental stress and genetic components in general, the problems involved in the investigation of the association of genetic parameters and the maple dieback are multifarious.

LITERATURE CITED

Anderson, F.O. 1989. Air pollution impact on Swedish forests - present evidence and future development. Environ. Monit. Assess. 12:29-38.

Bardy, J.-A. 1989. Acid deposition, summer drought and enhanced production of nitrate in forest soils; risk cofactors relative to forest decline. An additional hypothesis concerning the synergistical effects: the nitrous acid hypothesis. Environ. Lett. 10:681-686.

Beak Consultants Limited. 1989. Fertilization of healthy and declining sugar maple trees in Ontario. A Final Report For: Phytotoxicology Section, Air Resources Branch, Ontario Ministry of the Environment.

Bergmann, F., and F. Scholz. 1985. Effects of selection pressure by SO_2 pollution on genetic structures of Norway spruce (*Picea abies*). *In*: Population Genetics in Forestry - Lecture Notes in Biomathematics Vol 60. (Gregorius, H.R., ed.). pp 267-275. Springer-Verlag, N.Y.

Bergmann, F., and F. Scholz. 1987. The impact of air pollution on the genetic structure of Norway spruce. Silvae Genet. 36:80-83.

Bergmann, F., and F. Scholz. 1989. Selection effects of air pollution in Norway spruce (*Picea abies*) populations. *In* Genetic Effects of Air Pollutants in Forest Tree Populations. (F. Scholz, H.-R. Gregorius, and D. Rudin, eds) pp 144-160. Springer-Verlag, Berlin.

Cheliak, W.M., and J.A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Environ. Can., Can. For. Serv., Petawawa Natl. For. Inst. Inf. Rep. PI-X-42.

Geburek, Th., F. Scholz, W. Knabe, and A. Vornweg. 1987. Genetic studies by isozyme gene loci on tolerance and sensitivity in an air polluted *Pinus sylvestris* field trial. Silvae Genet. 36:49-53.

Guries, R.P., and F.T. Ledig. 1982. Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). Evolution (Lawrence Kans.), 36:387-402.

Hamburg, S.P., and C.V. Cogbill. 1988. Historical decline of red spruce populations and climatic warming. Nature, 331:428-431.

Hendershot, W.H., and A.R.C. Jones. 1989. Maple decline in Quebec: a discussion of possible causes and the use of fertilizers to limit damage. For. Chron. 65:280-287.

Huttunen, S. 1978. The effects of air pollution on provenances of Scots pine and Norway spruce in northern Finland. Silva Fenn. 12:1-16.

Hornbeck, J.W., and R.B. Smith. 1985. Documentation of red spruce growth decline. Can. J. For. Res. 15:1199-1201.

Hosking, G.P., and J.A. Hutcheson. 1988. Mountain beech (*Nothofagus solandri* var. *cliffortioides*) decline in the Kaweka Range, North Island, New Zealand. N. Z. J. Bot. 26:393-400.

Houston, D.B., and G.R. Stairs. 1973. Genetic control of sulfur dioxide and ozone tolerance in eastern white pine. For. Sci. 19:267-271.

Karnosky, D.F., P.C. Berrang, F. Scholz, and J.P. Bennett. 1989. Variation in and natural selection for air pollution tolerances in trees. *In* Genetic Effects of Air Pollutants in Forest Tree Populations. (F. Scholz, H.-R. Gregorius, and D. Rudin, eds) pp 29-37. Springer-Verlag, Berlin.

Knight, H.A. 1987. The pine decline. J. Forest. 85:25-28.

Kreibel H.B., and C. Leben. 1981. The impact of air pollution on the gene pool of eastern white pine. XVII IUFRO World Congr Kyoto, Japan. Proc Div 2, pp 185-189.

Lelliot, M. 1987. Inheritance of eight enzyme systems in sugar maple (*Acer saccharum* Marsh.). B.Sc.F. thesis, Lakehead University, Thunder Bay, Ont.

Linzon, S.N. 1985. Forest damage and acidic precipitation. The E.B. Eddy Distinguished Lecture Series, November, 1985. Faculty of Forestry, University of Toronto. Ontario.

Linzon, S.N. 1986. Activities and results of the terrestrial effects program: acid precipitation in Ontario study (APIOS). Water Air Soil Pollut. 31:295-305.

McIlveen, W.D., and D.L. McLaughlin. 1988. A survey to document the decline status of the sugar maple forest of Ontario: 1986/1987. Ontario Ministry of the Environment. Report No. ARB-158-88-Phyto.

McIlveen, W.D., S.T. Rutherford, and S.N. Linzon. 1986. A historical perspective of sugar maple decline within Ontario and outside of Ontario. Ontario Ministry of the Environment. Report No. ARB-141-86-Phyto.

McLaughlin, D.L., S.N. Linzon, D.E. Dimma, and W.D. McIlveen. 1985. Sugar maple decline in Ontario. Ontario Ministry of the Environment. Report No. ARB-144-85-Phyto.

Mitton, J.B., and J.M. Jeffers. 1989. The genetic consequences of mass selection for growth rate in Engelmann spruce. Silvae Genet. 38:6-12.

Morrison, I.K. 1984. Acid rain: a review of literature on acid deposition effects in forest ecosystems. For. Abstr. 45:483-505.

Mueller-Dombois, D. 1988. Towards a unifying theory for stand-level dieback. Geo J. 17:249-251.

Mulcahy, D.L. 1975. Differential mortality among cohorts in a population of *Acer saccharum* (Aceraceae) seedlings. Am. J. Bot. 62:422-426.

Müller-Starck, G. 1985. Genetic differences between "tolerant" and "sensitive" beeches (*Fagus sylvatica* L.) in an environmentally stressed adult forest stand. Silvae Genet. 34:241-247.

Müller-Starck, G. 1989. Genetic implications of environmental stress in adult forest stands of *Fagus sylvatica* L. *In* Genetic Effects of Air Pollutants in Forest Tree Populations. (F. Scholz, H.-R. Gregorius, and D. Rudin, eds) pp 128-142. Springer-Verlag, Berlin.

Perry, D.J., and P. Knowles. 1989. Allozyme variation in sugar maple at the northern limit of its range in Ontario, Canada. Can. J. For. Res. 19:509-514.

Pierre, M., and O. Queiroz. 1988. Air pollution by SO_2 amplifies the effects of water stress on enzymes and total soluble proteins of spruce needles. Physiol. Plant. 73:412-417.

Pimentel, R.A., and J.D. Smith. 1985. BIOΣTAT I. Sigma Soft, Placentia, CA.

Pitelka, L.F., and D.J. Raynal. 1989. Forest decline and acidic deposition. Ecology, 70:2-10.

Roberts, T.M., R.A. Skeffington, and L.W. Blank. 1989. Causes of type 1 spruce decline in Europe. Forestry, 62:179-222.

Scholz, F. 1981. Genecological aspects of air pollution effects on northern forests. Silva Fenn. 15:384-391.

Scholz, F. and F. Bergmann. 1984. Selection pressure by air pollution as studied by isozyme-gene-systems in Norway spruce exposed to sulphur dioxide. Silvae Genet. 33:238-241.

Scholz, F. and H. Venne. 1989. Structure and first results of a research program on ecological genetics of air pollution effects in Norway spruce. *In* Genetic Effects of Air Pollutants in Forest Tree Populations. (F. Scholz, H.-R. Gregorius, and D. Rudin, eds) pp 40-54. Springer-Verlag, Berlin.

Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (*Picea abies*) forest. Science, 244:776-783.

Schütt, P., and E.B. Cowling. 1985. Waldsterben, a general decline of forests in central Europe: Symptoms, development and possible causes. Plant Dis. 69:548-558.

Skelley, J.M. 1989. Forest decline versus tree decline - the pathological considerations. Environ. Monit. Assess. 12:23-27.

Wang, D., D.F. Karnosky, and F.H. Bormann. 1986. Effects of ambient ozone on the productivity of *Populus tremuloides* Michx. grown under field conditions. Can. J. For. Res. 16:47-55.

Willis, G.L., and M.S. Coffman. 1975. Composition, structure, and dynamics of climax stands of eastern hemlock and sugar maple in the Huron Mountains, Michigan. Tech. Bull. No. 13., Dept. of Forestry, Michigan Technological Univ., Houghton, Michigan.

Wisniewski, J., and J.D. Kinsman. 1988. pH and hydrogen ion deposition patterns in precipitation for the continental United States and Canada. Water Air Soil Pollut. 38:1-17.

Yeh, F.C., and C. Layton. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* spp. *latifolia*. Can. J. Genet. Cytol. 21:487-503.